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No effect of ornithine alphaketoglutarate on nitrogen excretion or urea synthesis rate in healthy male subjects

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Abstract

Background & aims: Ornithine alphaketoglutarate (OKG) is known for its anabolic and anticatabolic effects. Little is known about its effect on proteolysis. We studied urea formation and nitrogen excretion rates following OKG supplementation in healthy volunteers.

Methods: Sixteen male subjects were kept on a diet stable in protein (1 g/kg-d) and aimed at maintaining body weight. They received oral supplementation of OKG (10 g) or an isonitrogenous placebo (L-alanine) from days 4 to 6 in single daily doses in a randomised, blinded fashion. Urea kinetics were studied on day 4 from plasma urea enrichments following an oral [^{13}C]urea bolus. Nitrogen excretion was assessed from 24-h urine collections prior to supplementation, on day 4 and day 7.

Results: Mean urea rate of appearance was 380 ± 86 and 353 ± 120 $\mu\text{mol/kg}\cdot\text{h}$ in the OKG and placebo group, respectively ($p = 0.62$). There were no significant differences in 24-h nitrogen excretion data between groups, neither at separate time points, in overall values ($p = 0.40$) nor in development over time ($p = 0.53$).

Conclusions: A single oral dose of OKG did not affect urea production rate differently than the alanine placebo. OKG supplementation led to a temporary increase in nitrogen excretion, as did administration of placebo.

Introduction

L-ornithine alphaketoglutarate (OKG) is a nutritional supplement applied in both chronically^{255, 256} and acutely malnourished patients.²⁵⁷⁻²⁶¹ Apart from improving nutritional status, OKG supplementation has been suggested to stimulate immune function and to promote wound healing and tissue repair.^{258-260, 262}

Multiple studies have addressed the effect of OKG supplementation on protein metabolism in patients following burns,²⁵⁷⁻²⁶⁰ trauma²⁶¹ and surgery.^{85, 86, 265-269} In most, nitrogen balance studies were performed using 24-h urinary excretion of urea or nitrogen. All studies assessing intravenous OKG supplementation by nitrogen balance analysis reported an increase in nitrogen retention.^{86, 259-261, 265, 268, 269} Four studies performed muscle biopsies to assess total ribosome concentration or polyribosome number following OKG supplementation.^{85, 86, 263, 264} The number of ribosomes or polyribosomes is considered a proxy of protein synthesis capacity in skeletal muscle. In all studies, the decline in ribosome and polyribosome number seen in control patients was (at least in part) averted by OKG supplementation. Furthermore, OKG supplementation resulted in a less pronounced decline of muscle free glutamine concentrations.^{85, 86, 264} Finally, the effects of OKG on nitrogen balance, intramuscular glutamine concentrations and ribosome number were found to be interrelated.^{85, 86} The effect of OKG on protein metabolism in burn and surgery patients can thus be summarized as maintenance of protein synthesis capacity, possibly combined with decreased proteolysis, and resulting in increased nitrogen retention.

The effect of OKG on proteolysis, a characteristic feature of the metabolic response to injury or trauma,⁴ can only indirectly be ascertained from the results of the above-mentioned studies. After all, nitrogen intake, protein synthesis and proteolysis combined are all represented in nitrogen balance measurements. Proteolysis can be studied separately using stable isotopes studies. For these reasons, we chose to study the effect of OKG supplementation on urea production rate, urea being the final common pathway of amino acid breakdown.

Methods

a. Study subjects

Sixteen healthy, male volunteers participated in the study. All participants were in good health as determined by medical history, physical examination, analysis of blood cell count, routine blood biochemical profile and urinalysis. Exclusion criteria were smoking, consumption of five or more alcoholic drinks per week or more than six cups of caffeinated beverages per day, medication use or a body mass index < 18 or > 25 kg/m². Prior to inclusion, participants had to test negative for gastric *Helicobacter pylori* infection using the [¹³C]urea breath test.²⁶⁵ Written informed consent was obtained from all participants. The protocol was approved by the institution's Ethics Committee.

b. Dietary guidelines

Prior to the study, participants were interviewed at the Department of Nutrition and Dietetics for assessment of dietary intake and physical activity. Dietary guidelines were individually prescribed for a daily protein intake of 1 g/kg-d and aimed at maintaining body weight. Basal metabolic rate (BMR) was estimated using the Harris-Benedict formula¹³³ and a BMR factor of 1.4 was used to allow for habitual physical activity.

Participants were supplied with Dutch dietary tables²⁶⁶ and a kitchen scale (Terraillon, Versailles, France). They were instructed to adhere to the dietary instructions and to note and weigh dietary intake for the duration of the diet. Foodstuffs could be obtained from the institution's kitchen using vouchers and an especially prepared warm meal was served daily to participants.

c. Experimental design

The experimental protocol involved a total of 7 days, during which participants strictly complied with dietary guidelines. Subjects were randomised by the hospital pharmacy using balanced blocks of four. From days 4 to 6, single daily doses of either 10 g of OKG (Cetoran®, Chiesi SA, Courbevoie, France) or isonitrogenous placebo (8.3 g L-alanine, Nutricia Nederland BV, Zoetermeer, the Netherlands) were ingested under supervision. Both supplements were packed in identical containers in a blinded manner.

Subjects were free moving for the duration of the study and were encouraged to maintain their customary levels of physical activity but to refrain from excessive or competitive exercise. During the urea kinetics measurement day (day 4), subjects remained in the hospital for the major part of the day and commonly performed simple deskwork.

d. Measurements

Participants were seen daily for instructions, weight measurements and collection of dietary intake notes. Subjects were weighed on an electronic scale (SECA, Hamburg, Germany) to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm (DGI 250 D stadiometer, Winssen, the Netherlands).

Nitrogen excretion was determined in a total of three 24 hrs-collections, on days 2, 4 and 7. Urine was collected in plastic containers, kept refrigerated for the duration of collection and acidified with 10 mL 6 M HCl upon collection.

Urea kinetics were studied on day 4 following an overnight fast. Experiments started at 08:00 am. An i.v. needle was inserted into an antecubital vein and samples for background enrichment and plasma urea concentration were drawn. [¹³C]urea (99% isotopic purity; Campro, Veenendaal, the Netherlands), was weighed in 150 mg aliquots by the hospital pharmacy, dissolved in tap water, and administered as an oral dose on t = 0. Particular attention was paid to assure all tracer was ingested. Blood samples for plasma urea enrichments were drawn from the i.v. needle in 15-min intervals for the first 90 minutes and thereafter in 30-min intervals for a total duration of 6 h. Blood samples were collected in dry lithium heparinised tubes, placed on ice and centrifuged at 4 °C (2550 g, 10 min). Plasma was transferred into polyethylene cups and stored at -20 °C. Breath

samples were obtained in duplicate by blowing through a plastic straw into a glass test tube on $t = 0$ and 30 minutes after ingestion of the tracer. After the second breath sample had been obtained, the first dose of OKG or placebo supplement, dissolved in a glass of orange juice was administered. Thereafter, subjects received regular portions of simple, identical meals for the duration of 6 h. Subjects drank 100 mL water per hour.

e. Sample analysis

Total urinary nitrogen was determined using the Berthelot method.²⁴³ Urea concentration was determined enzymatically (Hitachi 747 autoanalyser, Roche, Mannheim, Germany) with a coefficient of variation of 6% ($n = 5$).

Urea in deproteinised blood samples was derivatised to 2-methoxypyrimidine and the [^{13}C] isotopic enrichment was determined by gas chromatography combustion isotope ratio mass spectrometry (GC-c-IRMS) (VG Optima, Micromass, Manchester, UK), as previously described.^{chapter 2} Measured ratios of peak areas were calibrated with a [^{13}C]urea enrichment standard curve and expressed as tracer to tracee molar ratio (coefficient of variation 0.002%). Measurement of [^{13}C] enrichment in exhaled air was performed by IRMS (BreathMAT plus, Finnigan, Bremen, Germany). The [$^{13}\text{CO}_2$] enrichments were measured against a reference CO_2 source, calibrated relative to Pee Dee Belmnite standard, and expressed as delta over baseline value ($\delta^{13}\text{C}$). A change in ^{13}C -enrichment $< 3.5\%$ following [^{13}C]urea ingestion was considered as negative for gastric *H. pylori* infection.²⁶⁷

f. Calculations

Total body water (TBW) was calculated using age, gender and anthropometric measurements.²⁶⁸ Urea pool was calculated from $\text{TBW} \times \text{plasma urea concentration}$. Urea rate of appearance (urea Ra) was calculated using single pool, log linear regression computations of the [^{13}C]urea enrichment curve as previously described.^{chapter 3} In the final analysis, urea Ra was calculated using [^{13}C]urea enrichments from $t = 150$ minutes onwards.

g. Statistical Analysis

Data were analysed using statistical software (SPSS 11.0, SPSS Inc, Chicago, IL, USA). Values are presented as mean \pm standard deviation (SD), unless stated otherwise. For comparisons between groups Student's *t* test was used. Changes in nitrogen excretion and body weight over time were analysed using multivariate analysis of variance (MANOVA) for repeated measurements. Differences were considered significant if $p < 0.05$ (two-tailed).

Results

a. Study subjects

Study subject characteristics were not different between groups (Table 1). All subjects completed the study. Energy and macronutrient intakes were similar between groups and are presented in Table 1. Mean (\pm SD) daily protein intake was 1.03 ± 0.16 g/kg·d. During the course of the study,

mean body weight showed a minimal, yet statistically significant decline from 73.8 to 73.5 kg ($p < 0.01$, multivariate analysis of variance (MANOVA) for repeated measurements). Supplementation allocation was found to have had no effect on body weight change ($p = 0.60$, MANOVA for repeated measurements).

Table 1 Characteristics and dietary intake of study participants

Variable	Controls n = 8	OKG n = 8	p-value
Age (years)	30.0 \pm 5.0	28.7 \pm 4.9	0.60
Body weight (kg)	77.0 \pm 7.5	70.4 \pm 7.7	0.10
Height (cm)	185.2 \pm 6.9	179.5 \pm 4.7	0.07
BMI (kg/m ²)	22.5 \pm 1.9	21.8 \pm 1.8	0.50
TBW (l)	44.7 \pm 4.0	42.7 \pm 2.8	0.26
Energy (kJ/d)	8,889 \pm 2,197	8,454 \pm 1,687	0.36
Protein (g/kg·d)	1.05 \pm 0.17	1.01 \pm 0.15	0.30
Fat (g/kg·d)	0.99 \pm 0.35	0.98 \pm 0.29	0.83
Carbohydrates (g/kg·d)	3.68 \pm 1.3	3.69 \pm 1.2	0.98

Values are means \pm standard deviation. OKG denotes ornithine alphaketoglutarate, BMI, body mass index and TBW, total body water.

b. Urea rate of appearance and urea pool

There was no difference in urea Ra between OKG and placebo groups ($380 \pm 86 \mu\text{mol/kg}\cdot\text{h}$ vs. $353 \pm 120 \mu\text{mol/kg}\cdot\text{h}$; $p = 0.62$). Urea pool calculated from the ¹³C-enrichment decay curve was in accordance with TBW * plasma urea concentration calculations ($219 \pm 81 \text{ mmol}$ vs. $210 \pm 51 \text{ mmol}$ respectively, $p = 0.63$).

c. Nitrogen excretion

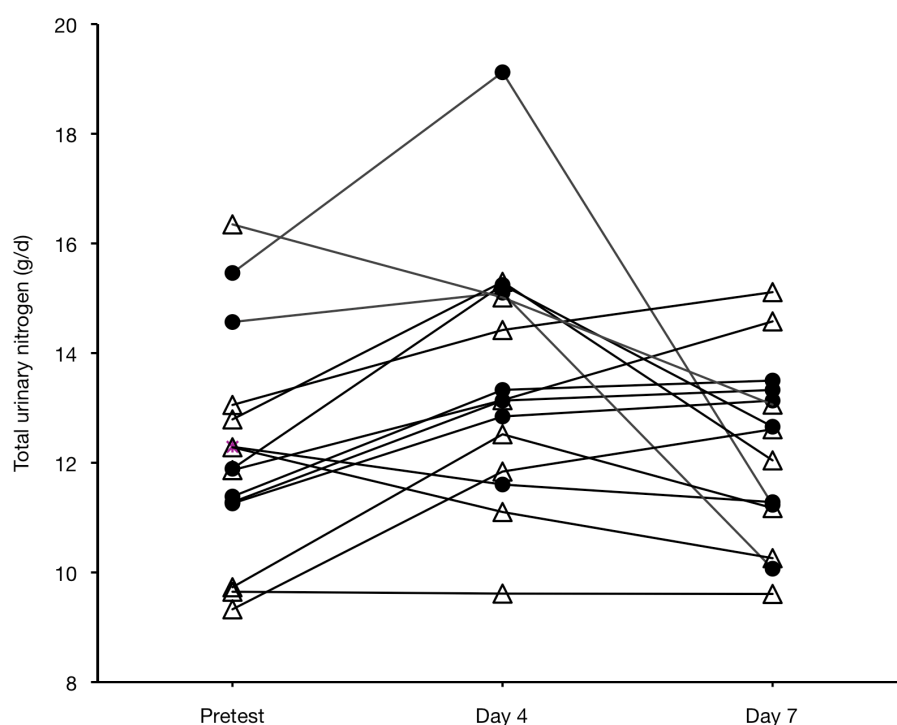
One 24-h urine collection of a study participant was lost. This subject (placebo group) was left out of nitrogen excretion analyses. Nitrogen excretion at separate time points did not differ between groups ($p = 0.73$ for pre-test, $p = 0.37$ for day 4 and $p = 0.91$ for day 7 respectively). MANOVA for repeated measurements found no significant differences in nitrogen excretion between OKG and placebo groups, neither in overall values ($p = 0.40$) nor in development over time ($p = 0.53$) (Table 2). Individual nitrogen excretion values are presented in Figure 1.

Additional analysis of urine collections on the first day of supplementation revealed significantly higher nitrogen excretion rates during the first 6 h immediately following ingestion of OKG or placebo as compared to the 18 h hereafter (9.1 ± 2 vs. $6.4 \pm 1 \text{ mg/kg}\cdot\text{h}$; $p < 0.05$ and 9.4 ± 2 vs. $6.7 \pm 1 \text{ mg/kg}\cdot\text{h}$; $p < 0.001$ in the OKG and placebo group, respectively).

Table 2 Nitrogen excretion

Variable	Pre-test	Day 4	Day 7	p-value
Control (n = 7)	12.6 ± 1.7	14.3 ± 2.5	12.2 ± 1.3	0.12
OKG (n = 8)	11.9 ± 2.3	12.9 ± 2.0	12.3 ± 1.9	0.63

Nitrogen excretion is expressed as g/d and presented as mean ± standard deviation. OKG denotes ornithine alphaketoglutarate. p-Values denote significance of overall effect of time on nitrogen excretion as assessed by multivariate analysis of variance (MANOVA).

Figure 1

Individual total urinary nitrogen excretion (g.d-1) from three 24-h collections (pretest, day 4 and day 7). OKG treated subjects (n = 8) are depicted by open triangles, subjects receiving placebo (n = 7) by closed circles.

Discussion

In this stable isotope study in healthy male subjects, a single dose of oral OKG did not affect urea production rate differently than an isonitrogenous placebo. Nitrogen excretion rate increased temporarily following OKG and placebo administration.

One previous study assessed the effect of OKG on urea production rate in seven healthy volunteers, albeit without control subjects.²⁶⁹ In this study, 4 grams of OKG were administered intravenously over 150 min. OKG is known to have immediate metabolic effects by stimulating the secretion of potent anabolic hormones as insulin and growth hormone.^{261, 270-272} Nevertheless, neither in the study by Eriksson et al.²⁶⁹ nor in our study an effect of OKG on urea production rate

could be demonstrated. One explanation might be that our protocol was too insensitive or too short to detect alterations in urea metabolism following OKG. The [^{13}C]urea bolus method produces one single value and does not yield continuous measurements, as is the case with primed, continuous infusion techniques. Secondly, the standard deviation of our measurements was much higher than assumed. Consequently, our number of volunteers was insufficient to detect a clinically significant effect. It may also be reasonable to assume that the alterations in whole body protein metabolism ascribed to OKG, i.e. improved nitrogen retention brought about by stimulation of protein synthesis capacity and diminished proteolysis, may follow only over the course of several days. From this point of view, our study would have benefited from a repeated tracer measurement following the supplementation period. It is noteworthy though, that in one of the few studies to perform stable isotope measurements regarding the effect of OKG, no evidence of decreased proteolysis was found.²⁶¹ Jeevanandam and Petersen found an increased rate of total protein turnover in trauma patients after 4 days of OKG supplementation. This was ascribed to an increased rate of protein synthesis and/or visceral proteolysis. Urinary nitrogen and 3 methyl-histidine excretion, both indicators of muscular proteolysis, were similar in OKG supplemented and control patients.

Another explanation for the findings of our study is that the beneficial effects of OKG on protein metabolism and nitrogen economy have all been reported in malnourished elderly or in patients suffering from burns or trauma.^{85, 257, 273, 274} The effects of OKG may be limited to conditions of metabolic stress, and for this reason may not have been present in our study. Our finding that OKG supplementation did not stimulate nitrogen retention over the course of three days is in accordance with this. Nitrogen excretion increased in the hours immediately following administration of OKG and placebo, indicating simple renal clearance of ingested nitrogen compounds.

Finally, subjects in our study were responsible for their own dietary intake. Despite individual instruction, especially prepared study meals and free provision of foodstuffs, adherence to the dietary guidelines varied between individuals. Especially caloric intake showed variances between individuals, as the main focus of the diet was a restricted and stable protein intake. On the whole, caloric intake did not completely match prescribed levels and mean body weight showed a minimal, yet statistically significant decline during the course of the study. The effect of a daily and individually varying caloric intake may have obscured possible effects of OKG supplementation on protein metabolism. However, we found this effect to be independent of supplementation group.

In conclusion, following oral supplementation of OKG, no immediate effect on urea production rate in healthy male study subjects could be demonstrated. Also, OKG supplementation did not increase nitrogen retention, as both OKG and placebo administration resulted in a temporary increase in nitrogen excretion. The previously reported positive effect on nitrogen economy and anticatabolic effect of OKG supplementation may only be present in critically ill patients or following a prolonged period of supplementation.